

BB



1. CONTRACT 2. AGREEMENT 3. DEED 4. WARRANTY 5. COVENANT 6. ASSURANCE 7. PROMISE 8. COMMITMENT 9. OBLIGATION 10. LIABILITY 11. RESPONSIBILITY 12. ACCOUNTABILITY 13. TRANSPARENCY 14. INTEGRITY 15. ETHICS 16. FAIRNESS 17. HONESTY 18. TRUTHFULNESS 19. ACCURACY 20. PRECISION 21. CLARITY 22. CONCISENESS 23. RELEVANCE 24. USEFULNESS 25. ADAPTABILITY 26. SCALABILITY 27. SECURITY 28. STABILITY 29. RELIABILITY 30. EFFICIENCY 31. EFFECTIVENESS 32. PRODUCTIVITY 33. PERFORMANCE 34. QUALITY 35. VALUE 36. IMPACT 37. INFLUENCE 38. POWER 39. AUTHORITY 40. RESPECT 41. DETERMINATION 42. RESOLVE 43. PERSEVERANCE 44. PERSISTENCE 45. ENDURANCE 46. STRENGTH 47. RESILIENCE 48. AGILITY 49. ADAPTABILITY 50. INNOVATION 51. CREATIVITY 52. IMAGINATION 53. IDEATION 54. PROBLEM-SOLVING 55. CRITICAL THINKING 56. ANALYTICAL SKILLS 57. LOGICAL REASONING 58. DECISION-MAKING 59. JUDGMENT 60. WISDOM 61. KNOWLEDGE 62. SKILL 63. KNOWLEDGE 64. EXPERIENCE 65. WISDOM 66. INSIGHT 67. UNDERSTANDING 68. EMPATHY 69. COMPASSION 70. SYMPATHY 71. CONCERN 72. CARE 73. ATTENTION 74. INTEREST 75. CURIOSITY 76. ENTHUSIASM 77. PASSION 78. ZEAL 79. ENTHUSIASM 80. ENERGY 81. VIGOR 82. SPRIT 83. MOOD 84. TEMPER 85. CHARACTER 86. PERSONALITY 87. IDENTITY 88. SELF 89. SOUL 90. HEART 91. MIND 92. BODY 93. SPirit 94. CONSCIENCE 95. CONSCIOUSNESS 96. AWARENESS 97. PERCEPTION 98. COGNITION 99. THOUGHT 100. FEELING 101. EMOTION 102. FEELING 103. EMOTION 104. FEELING 105. EMOTION 106. FEELING 107. EMOTION 108. FEELING 109. EMOTION 110. FEELING 111. EMOTION 112. FEELING 113. EMOTION 114. FEELING 115. EMOTION 116. FEELING 117. EMOTION 118. FEELING 119. EMOTION 120. FEELING 121. EMOTION 122. FEELING 123. EMOTION 124. FEELING 125. EMOTION 126. FEELING 127. EMOTION 128. FEELING 129. EMOTION 130. FEELING 131. EMOTION 132. FEELING 133. EMOTION 134. FEELING 135. EMOTION 136. FEELING 137. EMOTION 138. FEELING 139. EMOTION 140. FEELING 141. EMOTION 142. FEELING 143. EMOTION 144. FEELING 145. EMOTION 146. FEELING 147. EMOTION 148. FEELING 149. EMOTION 150. FEELING 151. EMOTION 152. FEELING 153. EMOTION 154. FEELING 155. EMOTION 156. FEELING 157. EMOTION 158. FEELING 159. EMOTION 160. FEELING 161. EMOTION 162. FEELING 163. EMOTION 164. FEELING 165. EMOTION 166. FEELING 167. EMOTION 168. FEELING 169. EMOTION 170. FEELING 171. EMOTION 172. FEELING 173. EMOTION 174. FEELING 175. EMOTION 176. FEELING 177. EMOTION 178. FEELING 179. EMOTION 180. FEELING 181. EMOTION 182. FEELING 183. EMOTION 184. FEELING 185. EMOTION 186. FEELING 187. EMOTION 188. FEELING 189. EMOTION 190. FEELING 191. EMOTION 192. FEELING 193. EMOTION 194. FEELING 195. EMOTION 196. FEELING 197. EMOTION 198. FEELING 199. EMOTION 200. FEELING 201. EMOTION 202. FEELING 203. EMOTION 204. FEELING 205. EMOTION 206. FEELING 207. EMOTION 208. FEELING 209. EMOTION 210. FEELING 211. EMOTION 212. FEELING 213. EMOTION 214. FEELING 215. EMOTION 216. FEELING 217. EMOTION 218. FEELING 219. EMOTION 220. FEELING 221. EMOTION 222. FEELING 223. EMOTION 224. FEELING 225. EMOTION 226. FEELING 227. EMOTION 228. FEELING 229. EMOTION 230. FEELING 231. EMOTION 232. FEELING 233. EMOTION 234. FEELING 235. EMOTION 236. FEELING 237. EMOTION 238. FEELING 239. EMOTION 240. FEELING 241. EMOTION 242. FEELING 243. EMOTION 244. FEELING 245. EMOTION 246. FEELING 247. EMOTION 248. FEELING 249. EMOTION 250. FEELING 251. EMOTION 252. FEELING 253. EMOTION 254. FEELING 255. EMOTION 256. FEELING 257. EMOTION 258. FEELING 259. EMOTION 260. FEELING 261. EMOTION 262. FEELING 263. EMOTION 264. FEELING 265. EMOTION 266. FEELING 267. EMOTION 268. FEELING 269. EMOTION 270. FEELING 271. EMOTION 272. FEELING 273. EMOTION 274. FEELING 275. EMOTION 276. FEELING 277. EMOTION 278. FEELING 279. EMOTION 280. FEELING 281. EMOTION 282. FEELING 283. EMOTION 284. FEELING 285. EMOTION 286. FEELING 287. EMOTION 288. FEELING 289. EMOTION 290. FEELING 291. EMOTION 292. FEELING 293. EMOTION 294. FEELING 295. EMOTION 296. FEELING 297. EMOTION 298. FEELING 299. EMOTION 300. FEELING 301. EMOTION 302. FEELING 303. EMOTION 304. FEELING 305. EMOTION 306. FEELING 307. EMOTION 308. FEELING 309. EMOTION 310

**PCT**

**(10) International Publication Number**  
**WO 02/02116 A2**

**(81) Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

**(84) Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**  
— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



**WO 02/02116 A2**

(57) **Abstract:** Methods are disclosed for treating cancer and inhibiting tumor growth by administering to a mammal in need thereof a therapeutically effective amount of 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-1H-pyrrole-2,5-dione, or a pharmaceutically acceptable salt or solvate thereof.

## THERAPEUTIC TREATMENT OF CANCER WITH A PROTEIN KINASE C INHIBITOR

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

5 The present invention is broadly directed to methods for treating cancer and inhibiting tumor growth with a protein kinase C (PKC) inhibitor. The present invention is particularly directed to the use of an isozyme selective PKC inhibitor for treating cancer and inhibiting tumor growth.

#### 2. Description of Related Art

Therapeutic treatments have been developed over the years to treat neoplasms. There are two major non-surgical approaches to treat neoplasms: 1) chemotherapy employing anti-neoplastic agents, and 2) radiation therapy. Other approaches include immunotherapies and antiangiogenic therapies. Anti-neoplastic agents and radiation cause cytotoxic effects, preferentially to tumor cells, and cause cell death.

Studies have shown that therapeutic radiation and most groups of anti-neoplastic agents assert their cytotoxic effects by activating programmed cell death or apoptosis. A balance between the activities of apoptotic and antiapoptotic intracellular signal transduction pathways is important in determining whether a cell will undergo apoptosis in response to the above-mentioned chemotherapy as well as radiation therapy.

PKC inhibitors have been proposed for cancer therapy (see, for example, U.S. 5,552,391) and it has been indicated that PKC activities exert antiapoptotic effects, especially in response to anti-neoplastic agents or radiation therapies. In particular, studies have shown that activation of PKC inhibits apoptosis induced by anti-neoplasm agents such as 1- $\beta$ -D- arabinofuranosylcytosine or Ara-c, etoposide or VP-16, *cis*-

diamminedichloroplatinum (II) or *cis*-platin, doxorubicin or adriamycin, 2- chloro-2-deoxyadenosine, 9- $\beta$ -D-arabinosyl-2-fluoroadenine, and glucocorticoids, and gamma irradiation therapy. There also have been indications that down regulation of PKC activities in tumor cells enhances apoptosis stimulated by anti-neoplastic agents. PKC activation has been shown to attenuate gamma-irradiation induced cell death (Blumberg PM, Acs P, Bhattacharyya DK, Lorenzo PS. Inhibitors of Protein Kinase C and related receptors for the lipophilic second-messenger sn-1,2-diacylglycerol. IN: J. S. Gutkind (ed.), Signaling Networks and Cell Cycle Control. Humana Press: Totowa, NJ, 2000; pps. 347-364).

- 10 Anticancer therapies, especially chemotherapies and radiation therapy , while frequently used, often produce some response in the malignant disease but are rarely curative. The importance of normal cells and tissues to support the growth of tumors have long been recognized. The observations of Van der Kolk (Pluda JM. Tumor-Associated Angiogenesis: Mechanisms, Clinical Implications, and Therapeutic Strategies. Seminars in Oncology 24:203-218 (1997) ("Pluda"), Jones (Norrby K. Angiogenesis: new aspects relating to its initiation and control. APMIS 105:417-437 (1997) ("Norrby") and Paget (Fox SB, Harris AL. Markers of tumor angiogenesis: clinical applications in prognosis and anti-angiogenic therapy. Investigational New Drugs 15:15-28 (1997) ("Fox 1")) more than 100 years ago documented this knowledge in the clinical science literature.
- 20 Fifty years ago, Algire and Chalkey (Risau W. What, if anything, is an angiogenic factor? Cancer and Metastasis Reviews 15:149-151 (1996) ("Risau")) reported that host vascular reactions could be elicited by growing tumors and described in detail the extent and tumor-specific nature of the induction of host capillaries by transplanted tumors. The central hypothesis of Algire and Chalkey was that vascular induction by solid tumors may
- 25 be the major, and possibly, the only distinguishing factor leading to tumor growth beyond normal tissue control levels. By the late 1960s, Folkman and his colleagues (Singh RK, Fidler IJ. Regulation of Tumor Angiogenesis by Organ-Specific Cytokines. Current Topics in Microbiology & Immunology 213:1-11 (1996) ("Singh") ; Iruela-Arispe ML, Dvorak HF. Angiogenesis: a dynamic balance of stimulators and inhibitors. Thrombosis and Haemostasis 78: 672-677 (1997) ("Iruela-Arispe"); Nguyen M. Angiogenic factors as tumor markers. Investigational New Drugs 15: 29-37 (1997) ("Nguyen")) had begun the
- 30

search for a tumor angiogenesis factor (TAF) and in 1971, Folkman proposed "antiangiogenesis" as a means of holding tumors in a non-vascularized dormant state (Toi M, Taniguchi T, Yamamoto Y, et al. Clinical Significance of the Determination of Angiogenic Factors. European Journal of Cancer 32A:2513-2519 (1996) ("Toi")).

- 5 Vascular endothelial growth factor (VEGF) is now recognized as a major angiogenesis factor in tumors. The intracellular signaling pathway of this endothelial cell specific growth factor involves the enzyme protein kinase C $\beta$  (PKC $\beta$ ), therefore potent selective inhibitors of PKC $\beta$  will block the mitogenic and migratory effects of VEGF on endothelial cells. Basic fibroblast growth factor and likely other angiogenic factors utilize
- 10 PKC $\beta$  in their intracellular signal transduction pathways. Small molecule inhibitors of PKC $\beta$  will, therefore in addition to enhancing malignant cell killing by chemotherapeutic agents and radiation therapies, be potent inhibitors of tumor angiogenesis and be widely applicable agents for the treatment of solid tumors.

- Most solid tumors increase in mass through the proliferation of malignant cells
- 15 and stromal cells including endothelial cells leading to formation of a tumor vasculature (Pluda). Since active angiogenesis is a critical component of the mass expansion of most solid tumors, this process is a valid target for therapy (Norrby). Angiogenesis, vasculature formation, during malignant growth is a complex process. Elucidation of the process has involved recognition of angiogenic stimuli such as hypoxia and nutrient
- 20 deprivation, recognition of angiogenic factors produced by malignant cells, fibroblasts and tumor infiltrating leukocytes and recognition that there may be a concomitant decrease in negative angiogenic regulators by the same three cell populations within the tumor for angiogenesis to occur (Norrby; Fox 1, Risau; Singh; Iruela-Arispe).
- Endogenous stimulators of angiogenesis, that is angiogenic factors, include vascular
- 25 endothelial growth factor (VEGF), transforming growth factors  $\alpha$  and  $\beta$  (TGF $\alpha$  and TGF $\beta$ ), acidic and basic fibroblast growth factor (a FGF and bFGF), epidermal growth factor (EGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP), angiogenin, pleiotropin, hepatocyte growth factor (HGF), interleukins-1,-
- 6,-8 (IL-1, IL-6, IL-8), placental growth factor, E-selectin, tumor necrosis factor- $\alpha$  (TNF-
- 30  $\alpha$ ), heparinase, angiopoietin, and hypoxia-inducible factor (Singh; Iruela-Arispe; Nguyen; Toi; Takebayashi Y, Akiyama S, Akiba S, et al. Clinicopathologic and Prognostic

Significance of an Angiogenic Factor, Thymidine Phosphorylase, in Human Colorectal Carcinoma. *J Natl Cancer Inst* 88:1110-71 (1996) ("Takebayashi 1"); Takebayashi Y, Yamada K, Miyadera K, et al. The Activity and Expression of Thymidine Phosphorylase in Human Solid Tumours. *European Journal of Cancer* 32A:1227-1232 (1996)

5 ("Takebayashi 2"); O'Brien TS, Fox SB, Dickinson AJ, et al. Expression of the Angiogenic Factor Thymidine Phosphorylase/Platelet-derived Endothelial Cell Growth Factor in Primary Bladder Cancers. *Cancer Research* 56:4799-4804 (1996) ("O'Brien"); Fox SB, Westwood M, Moghaddam A, et al. The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is up-regulated in breast cancer

10 epithelium and endothelium. *British Journal of Cancer* 73:275-280 (1996) ("Fox 2"); Griffiths L, Dachs G, Bicknell R, et al. The Influence of Oxygen Tension and pH on the Expression of Platelet-derived Endothelial Cell Growth Factor/Thymidine Phosphorylase in Human Breast Tumor Cells Grown *in Vitro* and *in Vivo*. *Cancer Research* 57:570-572 (1997) ("Griffiths"); Yoshiji H, Harris S, Thorgeirsson U. Vascular Endothelial Growth

15 Factor Is Essential for Initial but not Continued *in Vivo* Growth of Human Breast Carcinoma Cells. *Cancer Research* 57:3924-3928 (1997) ("Yoshiji"); Abedi H, Zachary I. Vascular Endothelial Growth Factor Stimulates Tyrosine Phosphorylation and Recruitment to New Focal Adhesions of Focal Adhesion Kinase and Paxillin in Endothelial Cells. *The Journal of Biological Chemistry* 272:15442-15451 (1997)

20 ("Abedi")). Endogenous negative angiogenic factors include vascular endothelial growth factor (VEGF), transforming growth factors  $\alpha$  and  $\beta$  (TGF $\alpha$  and TGF $\beta$ ), acidic and basic fibroblast growth factor (a FGF and bFGF), epidermal growth factor (EGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP), angiogenin, pleiotropin, hepatocyte growth factor (HGF), interleukins-1,-6,-8 (IL-1, IL-6,

25 IL-8), placental growth factor, E-selectin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), heparinase, angiopoietin, and hypoxia-inducible factor (id.). Endogenous negative regulators or inhibitors of angiogenesis include thrombospondin, platelet factor IV, interferon- $\alpha$ , interferon- $\beta$ , protamine, cartilage-derived inhibitors, angiostatin, endostatin, plasminogen activator inhibitor (PAI) and the tissue inhibitor of metalloproteinases (TIMPs) (Pluda;

30 Norrby; Singh; Abedi) Preclinical and clinical studies have shown that malignant cells in culture and tumors *in vivo* can and, most often, do express an array of angiogenesis

stimulators and negative regulators. Clearly, angiogenesis is a highly complex and closely regulated process and it is not surprising that vasculature in malignant masses is often poorly formed, irregular, lacking complete structure and inadequate to feed the tissue (Anand-Apte B, Bao L, Smith R, et al. A review of tissue inhibitor of metalloproteinases-3 (TIMP-3) and experimental analysis of its effect on primary tumor growth. *Biochem. Cell Biol.* 74: 853-862 (1996); Fukumura D, Yuan F, Monsky WL, et al. Effect of host microenvironment on the micro-circulation of human colon adenocarcinoma. *American Journal of Pathology* 151: 679-688 (1997); Less JR, Posner MC, Skalak TC, et al. Geometric resistance and microvascular network architecture of human colorectal carcinoma. *Microcirculation* 4: 25-33 (1997)). The combination of antiangiogenic agents with standard therapies appears to be synergistic (Jain RK. The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 4: 1-23 (1997)).

The most clear-cut, direct-acting, most frequently found angiogenic factor in cancer patients is vascular endothelial growth factor (VEGF) (Pluda; Norrby; Fox 2; Risau; Jain RK. The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. *Microcirculation* 4: 1-23 (1997)). VEGF expression has been positively associated with primary breast cancer, brain tumors, cervical neoplasias, lung cancer, stomach, colon cancer and others. Furthermore, the up-regulation of the VEGF receptors, flt-1 and KDR, has been observed in tumor-associated endothelial cells in a variety of tumors including breast cancer, brain tumors, kidney tumors, bladder cancer, ovarian cancer and colon cancer. The signal transduction pathways of the KDR/Flk-1 and Flt-1 receptors include tyrosine phosphorylation, activation of PLC $\gamma$ , diacylglycerol generation, and PI-3 kinase with downstream activation of protein kinase C (PKC) and activation of the MAP kinase pathway (Teicher BA, ed. *Antiangiogenic Agents in Cancer Therapy*. New Jersey: The Humana Press, Inc., 1999, Vol. 4; Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, and King GL, Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth, *Journal of Clinical Investigation* 98: 2018-2026 (1996); Guo D, Jia Q, Song HY, Warren RS, and Donner DB, Vascular endothelial cell growth factor promotes tyrosine phosphorylation

of mediators of signal transduction that contain SH2 domains. Association with endothelial cell proliferation, Journal of Biological Chemistry 270: 6729-6733 (1995); Sawano A, Takahashi T, Yamaguchi S and Shibuya M, The phosphorylated 1169-tyrosine containing region of flt-1 kinase (VEGFR-1) is a major binding site for PLCgamma, Biochemical & Biophysical Research Communications 238: 487-491 (1997)). This intracellular signal transduction pathway may be common, at least in part, for most angiogenic factors.

Protein kinase C is a gene family consisting of at least 12 isoforms (Mohammadi M, Dikic I, Sorokin A, Burgess WH, Jaye M, and Schlessinger J, Identification of six novel autophosphorylation sites on fibroblast growth factor receptor 1 and elucidation of their importance in receptor activation and signal transduction, Molecular & Cellular Biology 16, 977-989 (1996)). Based on differing substrate specificity, activator requirements and subcellular compartmentalization, it is hypothesized that activation of individual protein kinase C isoforms preferentially elicits specific cellular responses (id.).

There is a need in the art to develop therapeutic agents which enhance the apoptotic signal transduction pathways in malignant cells and inhibit the signal transduction pathways of angiogenic factors in tumors, and thereby are useful in treating cancer and inhibiting tumor growth.

## SUMMARY OF INVENTION

It is an object of the invention to provide methods for treating cancer.

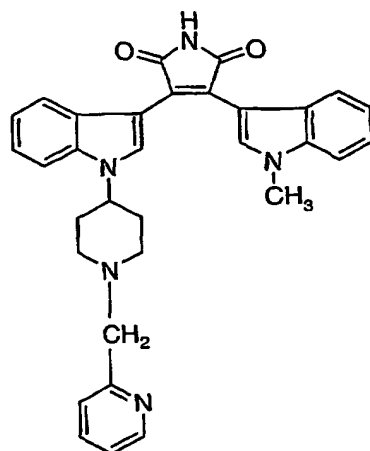
It is another object of the invention to provide methods for inhibiting tumor growth.

These and other objects of the invention are provided by one or more of the embodiments described below.

In one embodiment of the invention there is provided a method for treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione (which is the compound of Formula I)

-7-

or a pharmaceutically acceptable salt or solvate thereof.



Formula I

In another embodiment of the invention there is provided a method for inhibiting  
5 tumor growth comprising administering to a mammal in need thereof a therapeutically  
effective amount of a compound of Formula I.

Another embodiment of the invention provides for the use of a compound of  
Formula I or a pharmaceutically acceptable salt or solvate thereof for the manufacture of  
a medicament for the treatment of cancer.

10 Still another embodiment of the invention provides for the use of a compound of  
Formula I or a pharmaceutically acceptable salt or solvate thereof for the manufacture of  
a medicament for inhibiting tumor growth.

Still another embodiment of the invention provides for the use of a compound of  
Formula I or a pharmaceutically acceptable salt or solvate thereof for the manufacture of  
15 a medicament for the treatment of cancer.

Preferred embodiments of the invention provide for such methods and uses  
wherein the cancer being treated is lung, bladder, breast, colon, brain, cervical, stomach  
or kidney cancer.

Other preferred embodiments of the invention provide for such methods and uses  
20 for inhibiting the growth of lung, bladder, breast, colon, brain, cervical, stomach or  
kidney tumors.

Other preferred embodiments of the invention provide for such methods and uses  
for inhibiting the growth of human SW2 small lung carcinoma, human T98G



glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma tumors.

5       Special embodiments of the invention provide for such methods and uses wherein the cancer being treated is human SW2 small lung carcinoma, human T98G glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1  
10   breast carcinoma.

The present invention provides the art with a method for increasing apoptotic effects in malignant cells and inhibiting the signal transduction of angiogenic factors in tumors, and is thus effective in treating cancer and inhibiting tumor growth.

15       **DETAILED DESCRIPTION OF THE INVENTION**

It is a discovery of the present invention that use of a compound of Formula I, reduces or inhibits anti-apoptotic effects in a malignant cell and inhibits the intracellular signal transduction of angiogenic factors. Consequently, this compound can be used to  
20   treat cancer and inhibit tumor growth.

This compound may be prepared by methods known in the art (see, for example, U.S. Patent No. 5,668,152).

Because it contains a basic moiety, the compound of Formula I can also exist as pharmaceutically acceptable acid addition salts. Acids commonly employed to form  
25   such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, mono-  
30   hydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate,

maleate, 2-butyne-1,4-dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate,  $\beta$ -hydroxybutyrate, glycolate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Particularly the hydrochloric and mesylate salts are used.

In addition to pharmaceutically acceptable salts, other salts also can exist. They may serve as intermediates in the purification of the compound of Formula I, in the preparation of other salts, or in the identification and characterization of the compound or intermediates.

The pharmaceutically acceptable salts of the compound of Formula I can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Neoplasia is characterized by abnormal growth of cells which often results in the invasion of normal tissues, *e.g.*, primary tumors or the spread to distant organs, *e.g.*, metastasis. Any neoplastic growth may be treated by the methods of the present invention. Such neoplastic growth includes but not limited to primary tumors, primary tumors that are incompletely removed by surgical techniques, primary tumors which have been adequately treated but which are at high risk to develop a metastatic disease subsequently, and an established metastatic disease.

Specifically, the PKC inhibitor of Formula I above can be used to treat cancer and to inhibit tumor growth.

The following examples are provided merely to further illustrate the present invention. The scope of the present invention is not to be construed as merely consisting of the following examples. In each of the following examples, the compound of Formula I is administered as the dihydrochloride salt (the "Compound"), and the amounts administered are given in terms amounts of the dihydrochloride salt.

The primary pharmacologic activity of the Compound is the inhibition of vascular endothelial growth factor (VEGF)-induced angiogenesis and basic fibroblast growth

-10-

factor-induced angiogenesis associated with neoplastic disease. Three assay systems demonstrate this action of the molecule: 1.) the Compound is a potent inhibitor of VEGF-stimulated proliferation of HUVEC cells in culture with an  $IC_{50}$  of 150 nM upon 72 hrs. exposure to the compound; 2.) the Compound is a highly effective inhibitor of VEGF-induced neoangiogenesis in the rat corneal micropocket when administered orally to the animals for 10 days; 3.) the Compound decreases to one-half to one-third the number of intratumoral blood vessels in the human SW2 small cell lung carcinoma grown as a xenograft in nude mice when administered orally for two weeks.

The treatment of neoplastic disease requires eradication of the primary and metastatic disease. The Compound demonstrated activity in each of ten tumors tested. The murine Lewis lung carcinoma or human T98G glioblastoma multiforme, human SW2 small cell lung carcinoma, human Calu-6 non-small cell lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human HS746T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma was implanted in male C57Bl mice or male or female nude mice and the tumor-bearing animals were treated with either the Compound alone or with standard anticancer chemotherapy (paclitaxel, carboplatin, gemcitabine, BCNU, 5-fluorouracil, irinotecan or cisplatin) or with fractionated radiation therapy.

Specifically, for most experiments, tumor cells prepared from a brief of donor tumors ( $5 \times 10^6$  cells) were implanted subcutaneously in a hind-leg of mice (Charles River). Intracranial tumors were implanted by injecting  $10^4$  T98G cells in 1.5 microliters. Intraperitoneal tumors were implanted by injecting  $5 \times 10^6$  SKOV-3 tumor cells intraperitoneally into female nude mice. Treatment with the Compound (3, 10 or 30 mg/kg) administered orally twice per day for schedules lasting 2 weeks or more. Each standard chemotherapeutic agent was administered in a full murine dose regimen for that agent. Each treatment group as well as a group of untreated control animals consisted of 5 animals per group in each experiment. Subcutaneous tumor response was monitored by tumor volume measurement performed twice per week over the course of 60-120 days. For the survival experiments, long-term survivors (cures) were determined at 120 days post tumor implantation. Body weights were measured as a general measure of toxicity.

The subcutaneous tumor data were analyzed by determining the mean tumor volume for each treatment group over the course of the experiment and calculating the tumor growth delay as the difference in days for the treatment versus the control tumors to reach a volume of 500 and 1000 mm<sup>3</sup>.

5

#### Growth Inhibition of Endothelial and Malignant Cells by the Compound

Human Umbilical Vein Endothelial Cell Culture Assay. HUVEC cells in basal medium were stimulated to proliferate by exposure to human VEGF (20 ng/ml). Human tumor cells were grown in media with 2% fetal bovine serum. When the Compound was added to the cultures for 72 hours, the proliferation of the VEGF-stimulated HUVEC cells was profoundly inhibited by 0.6  $\mu$ M of the compound. The IC<sub>50</sub> for the HUVEC assay was 150 nM of the Compound (as shown in Table 1). The Compound was growth inhibitory at concentrations of 3 to >85  $\mu$ M in eight human tumor cell lines exposed to the compound for the same period of time assuming (Concentration times Time) (as shown in Table 1).

15

#### Rat Corneal Micropocket Assay

The cornea is normally an avascular tissue. Surgical implantation of a small filter disc impregnated with vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) into the cornea will result in robust neoangiogenesis that is quantifiable in 7 to 10 days. Administration of the Compound orally twice per day on days 1 through 10 post surgical implant of VEGF or bFGF impregnated filters resulted in markedly decreased vascular growth in the cornea. A dose of 10 mg/kg of the Compound decreased vascular growth to about one-half of the VEGF stimulated controls; while a dose of 30 mg/kg of the Compound decreased vascular growth to the level of the unstimulated surgical control in the VEGF-stimulated corneas and to a level of one-third of control in the bFGF-stimulated corneas. Results for VEGF are shown in Table 2, and for bFGF in Table 3.

25

#### Intratumoral Vessel Count Assay.

30

Nude mice bearing human SW2 small cell lung carcinoma, human Calu-6 non-small cell lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon

-12-

carcinoma, human CaKi1 renal cell carcinoma, human HS746T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma growing as a subcutaneous xenograft on the thigh of the animals were treated with the Compound twice daily on days 14 through 30 post tumor cell implantation. On day 31 tumors were collected, preserved in 10% phosphate buffered formalin and 5 mm thick sections were immunohistochemically stained for expression of endothelial specific markers, either Factor VIII, CD31 or CD105. The number of intratumoral vessels in the samples was quantified by counting stained regions in 10 high power microscope fields (200x). The results are shown in Tables 4 to 11.

#### Tumor Growth Delay in Human Xenografts

The Compound, administered orally twice per day for 2 weeks, has demonstrated activity in a variety of human tumor xenografts grown in nude mice (Tables 12 to 19).

In nine tumor lines grown in vivo in mice, the tumor growth delay produced by the Compound (30 mg/kg) administered twice per day orally for 2 weeks compared very favorably with the tumor growth delay produced by standard anticancer agents administered on a normal full murine dose-schedule (Table 20). In Table 5, RADS is 3 Gray x 10.

**TABLE 1**

**GROWTH INHIBITION OF ENDOTHELIAL AND MALIGNANT CELLS BY  
THE COMPOUND**

Cell Type	Compound IC <sub>50</sub> , $\mu$ M
HUVEC ENDOTHELIAL	0.15
Calu-6 NON-SMALL CELL LUNG CA	4.2
SW-2 SMALL CELL LUNG CA	3.5
MX-1 BREAST CA	3.3
CaKi RENAL CELL CA	3.3

-13-

Cell Type	Compound IC <sub>50</sub> , $\mu$ M
SKOV3 OVARIAN CA	6.3
HT29 COLON CA	8.5
HS746T GASTRIC CA	29
Hep3B HEPATOCELLULAR CA	>85

**TABLE 2**

5 **RAT CORNEAL MICROPOCKET VEGF INDUCED ANGIOGENESIS:  
RESPONSE TO THE COMPOUND, po, 2x d1-10**

	Vascular Area (pixels)	SEM
Control	756	412
VEGF	4171	682
VEGF and Compound (10mg/kg)	2052	593
VEGF and Compound (30 mg/kg)	768	501

**TABLE 3**

10 **RAT CORNEAL MICROPOCKET bFGF INDUCED ANGIOGENESIS:  
RESPONSE TO THE COMPOUND, po, 2x d1-10**

	Vascular Area (pixels)	SEM
Control	756	412
bFGF	4195	714
bFGF and Compound (30mg/kg)	1646	918

-14-

**TABLE 4****HUMAN CALU-6 NON-SMALL CELL LUNG CA**

5

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	17	8	20	4.5
Compound (30mg/kg) po 2 x d 14-30	8	3.5	10	4

**TABLE 5**

10

**HUMAN CaKi1 RENAL CELL CARCINOMA**

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	10.6	1.2	11	1.7
Compound (30mg/kg) po 2 x d 14-30	1.2	0.3	2	0.4

**TABLE 6**

15

**HUMAN HT-29 COLON CARCINOMA**

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	9.6	1.1	11.2	1.4
Compound (30mg/kg) po 2 x d 14-30	3.2	0.7	4.6	1.1

-15-

**TABLE 7****HUMAN HS746T GASTRIC CARCINOMA**

5

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	19	6	11	3
Compound (30mg/kg) po 2 x d 14-30	15	8	7	3

**TABLE 8****HUMAN Hep3B HEPATOCELLULAR CARCINOMA**

10

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	7	3	4	1
Compound (30mg/kg) po 2 x d 14-30	3	2	1.4	0.5

**TABLE 9****HUMAN MX-1 BREAST CARCINOMA**

15

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	26	4	7	3
Compound (30mg/kg) po 2 x d 14-30	17	4	4	2



-16-

**TABLE 10**  
**HUMAN SKOV-3 OVARIAN CARCINOMA**

Treatment Group	CD31		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	5	2	4	2
Compound (30mg/kg) po 2 x d 14-30	2	1	1	1

5

**TABLE 11**  
**HUMAN SW2 SMALL CELL LUNG CANCER**

Treatment Group	Factor VIII		CD105	
	Mean No. Intratumoral Vessels Per Field	SEM	Mean No. Intratumoral Vessels Per Field	SEM
Control	50	7	80	8
Compound (3 mg/kg) 2 x d 14-30	40	9	64	10
Compound (10 mg/kg) 2 x d 14-30	38	8	42	7
Compound (30mg/kg) 2 x d 14-30	27	7	25	6

10

**TABLE 12**  
**RESPONSE OF Calu-6 TO THE COMPOUND AS A SINGLE AGENT**

Dose (mg/kg) 2 x per day x 14	Tumor Growth Delay (days)
3	4.4
10	6.3
30	8.8

**TABLE 13****RESPONSE OF SW2 TO THE COMPOUND AS A SINGLE AGENT**

Dose (mg/kg) 2 x per day x 14	Tumor Growth Delay (days)
3	7.4
10	7.5
30	9.7

5

**TABLE 14****RESPONSE OF Caki-1 TO THE COMPOUND AS A SINGLE AGENT**

Dose (mg/kg) 2 x per day x 14	Tumor Growth Delay (days)
3	9.7
10	14.2
30	15

10

**TABLE 15****RESPONSE OF HS746T TO THE COMPOUND AS A SINGLE AGENT**

Dose (mg/kg) 2 x per day x 14	Tumor Growth Delay (days)
3	6.5
10	9.2
30	15.2

**TABLE 16**

15

**RESPONSE OF Hep3B TO THE COMPOUND AS A SINGLE AGENT**

Dose (mg/kg) 2 x per day x 14	Tumor Growth Delay (days)
3	5.2
10	20.3
30	21.8

**TABLE 17****RESPONSE OF T98G TO THE COMPOUND AS A SINGLE AGENT**

5

<b>Dose (mg/kg) 2 x per day x 14</b>	<b>Tumor Growth Delay (days)</b>
10	6
30	8

**TABLE 18****RESPONSE OF HT-29 TO THE COMPOUND AS A SINGLE AGENT**

10

<b>Dose (mg/kg) 2 x per day x 14</b>	<b>Tumor Growth Delay (days)</b>
10	10.1
30	14.5

**TABLE 19****RESPONSE OF MX-1 TO THE COMPOUND AS A SINGLE AGENT**

15

<b>Dose (mg/kg) 2 x per day x 14</b>	<b>Tumor Growth Delay (days)</b>
10	15.3
30	21.3

**TABLE 20****TUMOR GROWTH DELAY BY THE COMPOUND COMPARED WITH STANDARD AGENTS**

20

	<b>Tumor Growth Delay (days)</b>							
<b>Tumor Line</b>	<b>Compound</b>	<b>Paclitaxel</b>	<b>Carboplatin</b>	<b>Gemcitabine</b>	<b>BCNU</b>	<b>5-FU</b>	<b>Cisplatin</b>	<b>Rads</b>
Lewis Lung Ca	6	4.5	5	7				
T98G	9				4.5			
Calu-6 NSCLC	11	6	5					
SW2 Small Cell Lung 10	10	25	5					

Tumor Line	<u>Tumor Growth Delay (days)</u>							
	Compound	Paclitaxel	Carboplatin	Gemcitabine	BCNU	5-FU	Cisplatin	Rads
HT-29 Colon Ca	15					7.5	6	
MX-1 Breast Ca	20	37	10					
CaKil Renal Ca	15			19.5				18.5
HS746T Gastric Ca	15.2			14.9		3		
Hep3B Liver Ca	25.2			10.9		8.9		

In addition, when administered to animals bearing intracranial human T98G glioblastoma multiforme, the mean survival time of the animals treated with the Compound (30 mg/kg, po) was 72 days. The mean survival time for animals treated with BCNU alone in this study was 42 days and the mean survival time for the untreated control animals was 39 days.

Survival was also used as an endpoint for studies with animals bearing intraperitoneally implanted human SKOV-3 ovarian carcinoma. The mean survival time for animals treated with the Compound (30 mg/kg, po) was 73 days. The mean survival times for animals in these studies treated with paclitaxel was 84 days and for animals treated with carboplatin was 45 days. In these studies the untreated control animals survived 40 days.

The PKC inhibitor may be administered chronically or semi-chronically, over a period of from about 2 weeks to about 5 years.

One skilled in the art will recognize that the amount of PKC inhibitor to be administered in accordance with the present invention, that is the therapeutically effective amount, is that amount sufficient to produce an anti-neoplastic effect, to induce apoptosis or cell death, and/or to maintain an antiangiogenic effect. Such amount may vary, among other factors, depending upon the size and the type of neoplasia, the concentration of the compound in the therapeutic formulation, the timing of the

administration of the PKC inhibitor relative to the other therapies, and the age, size and condition of the patient.

Generally, an amount of protein kinase C inhibitor to be administered is decided on a case by case basis by the attending physician. As a guideline, the extent of the neoplasia, the body weight, and the age of the patient will be considered, among other factors, when setting an appropriate dose.

Generally, a suitable dose is one that results in a concentration of the protein kinase C inhibitor at the site of tumor cells in the range of 50 nM to 50  $\mu$ M, and more usually from 100 nM to 10  $\mu$ M. It is expected that serum concentrations of 250 nM to 250  $\mu$ M should be sufficient in most circumstances.

To obtain these treatment concentrations, a patient in need of treatment will be administered between about 0.5 mg per day per kg of body weight and about 10 mg per day per kg. Usually, not more than about 3 mg per day per kg of body weight of protein kinase C inhibitor should be needed. As noted above, the above amounts may vary on a case-by-case basis.

The compound of the present invention is preferably formulated prior to administration. Suitable pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. In making the compositions suitable for use in the method of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders for either oral or topical application.

Some examples of suitable carriers, excipient, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphates, alginate, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc,

magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 mg to about 3 g, more usually about 64 mg of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the severity of the condition to be treated, the choice of compound to be administered and the chosen route of administration. Therefore, the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, most of which may be administered orally, the compound used in the method of the present invention also may be administered topically. Topical formulations include ointments, creams and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon

-22-

formation of the emulsion, the active ingredient (compound) customarily is added in an amount to achieve the desired concentration.

Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound incorporated into a topical formulation is not critical; the concentration should be within a range sufficient to permit ready application of the formulation to the affected tissue area in an amount which will deliver the desired amount of compound to the desired treatment site.

The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to the effected tissue in an amount affording from about 1 to about 500  $\mu\text{g}$  compound per  $\text{cm}^2$  of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300  $\mu\text{g}/\text{cm}^2$ , more preferably, from about 50 to about 200  $\mu\text{g}/\text{cm}^2$ , and, most preferably, from about 60 to about 100  $\mu\text{g}/\text{cm}^2$ .

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

#### Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active agent	250
starch, dried	200
magnesium stearate	10
Total	<hr/> 460 mg

25

-23-

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

5 A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active agent	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	<hr/> 665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

10 Tablets each containing 60 mg of active ingredient are made as follows:

	Quantity (mg/tablet)
Active agent	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
Total	<hr/> 150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve.

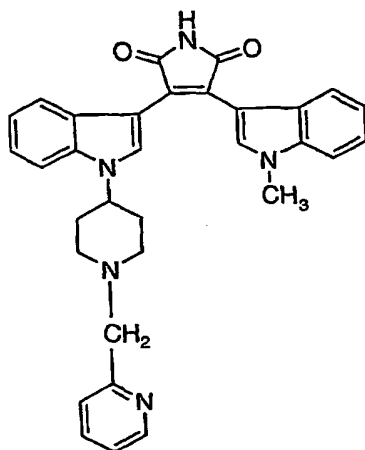


The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

5 The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since they are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.

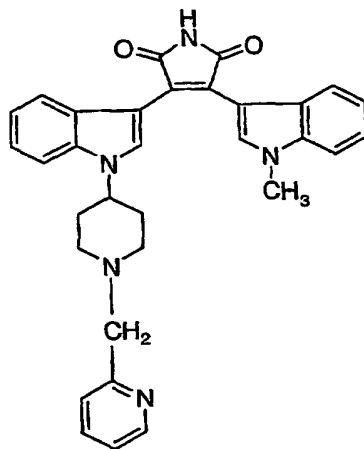
**CLAIMS:**

1. A method for treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the formula
- 5



or a pharmaceutically acceptable salt or solvate thereof.

2. A method for inhibiting tumor growth comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the formula

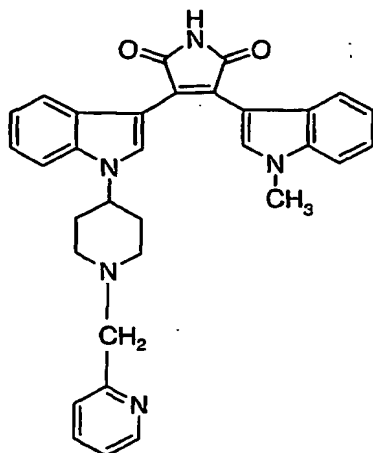


10

or a pharmaceutically acceptable salt or solvate thereof.

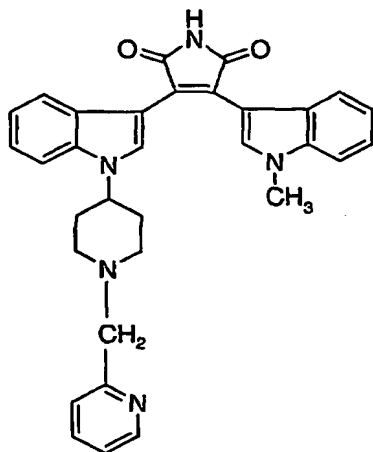
-26-

## 3. The use of a compound of the formula



or a pharmaceutically acceptable salt or solvate thereof for the manufacture of a  
5 medicament for the treatment of cancer.

## 4. The use of a compound of the formula



or a pharmaceutically acceptable salt or solvate thereof, for the manufacture of a  
10 medicament for inhibiting tumor growth.

5. A method according to claim 1 wherein the cancer being treated is lung, bladder, breast, colon, brain, cervical, stomach or kidney cancer.

6. A method according to claim 2 for inhibiting the growth of a lung, bladder, breast, colon, brain, cervical, stomach or kidney tumor.

-27-

7. A use according to claim 3 wherein the cancer being treated is lung, bladder, breast, colon, brain, cervical, stomach or kidney cancer.
8. A use according to claim 4 for inhibiting the growth of a lung, bladder, breast, colon, brain, cervical, stomach or kidney tumor.
- 5 9. A method according to claim 5 wherein the cancer being treated is human SW2 small lung carcinoma, human T98G glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma.
- 10 10. A method according to claim 6 for inhibiting the growth of a human SW2 small lung carcinoma, human T98G glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma tumor.
- 15 11. A use according to claim 7 wherein the cancer being treated is human SW2 small lung carcinoma, human T98G glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma.
- 20 12. A use according to claim 8 for inhibiting the growth of a human SW2 small lung carcinoma, human T98G glioblastoma multiforme, human Calu-6 non-small lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, human CaKi1 renal cell carcinoma, human H5747T gastric cancer, human Hep3B hepatocellular carcinoma or human MX-1 breast carcinoma tumor.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
10 January 2002 (10.01.2002)

PCT

(10) International Publication Number  
**WO 02/02116 A3**

- (51) International Patent Classification<sup>7</sup>: **A61K 31/4468**, **A61P 35/00**
- (21) International Application Number: **PCT/US01/16502**
- (22) International Filing Date: **28 June 2001 (28.06.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:  
**60/215,172** **29 June 2000 (29.06.2000)** **US**
- (71) Applicant (for all designated States except US): **ELI LILLY AND COMPANY [US/US]**; Lilly Corporate Center, Indianapolis, IN 46285 (US).
- (72) Inventors; and  
(75) Inventors/Applicants (for US only): **TEICHER, Beverly, Ann [US/US]**; 1357 Worchester Drive, Carmel, IN 46033 (US). **WAYS, Douglas, Kirk [US/US]**; 11 Davis Court, Bridgewater, NJ 08836 (US).
- (74) Agents: **DARKES, Paul, R. et al.**; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).
- (81) Designated States (national): **AE. AG. AL. AM. AT. AU. AZ. BA. BB. BG. BR. BY. BZ. CA. CH. CN. CO. CR. CU. CZ. DE. DK. DM. DZ. EC. EE. ES. FI. GB. GD. GE. GH. GM. HR. HU. ID. IL. IN. IS. JP. KE. KG. KP. KR. KZ. LC. LK. LR. LS. LT. LU. LV. MA. MD. MG. MK. MN. MW. MX. MZ. NO. NZ. PL. PT. RO. RU. SD. SE. SG. SI. SK. SL. TJ. TM. TR. TT. TZ. UA. UG. US. UZ. VN. YU. ZA. ZW.**
- (84) Designated States (regional): **ARIPO** patent (GH. GM. KE. LS. MW. MZ. SD. SL. SZ. TZ. UG. ZW). **Eurasian** patent (AM. AZ. BY. KG. KZ. MD. RU. TJ. TM). **European** patent (AT. BE. CH. CY. DE. DK. ES. FI. FR. GB. GR. IE. IT. LU. MC. NL. PT. SE. TR). **OAPI** patent (BF. BJ. CF. CG. CI. CM. GA. GN. GW. ML. MR. NE. SN. TD. TG).
- Published:**  
— with international search report  
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:  
**23 May 2002**
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/02116 A3

(54) Title: THERAPEUTIC TREATMENT OF CANCER WITH A PROTEIN KINASE C INHIBITOR

(57) Abstract: Methods are disclosed for treating cancer and inhibiting tumor growth by administering to a mammal in need thereof a therapeutically effective amount of 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-1H-pyrrole-2,5-dione, or a pharmaceutically acceptable salt or solvate thereof.

## INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 01/16502

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K31/4468 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 01 30331 A (CAMERON NORMAN EUGENE ;WAYS DOUGLAS KIRK (US); LILLY CO ELI (US)) 3 May 2001 (2001-05-03) claims 1-17	1-12
E	WO 02 02094 A (TEICHER BEVERLY ANN ;WAYS DOUGLAS KIRK (US); LILLY CO ELI (US)) 10 January 2002 (2002-01-10) claims 1-15	1-12
X	WO 95 17182 A (LILLY CO ELI) 29 June 1995 (1995-06-29) page 21, line 21 - line 28; claims 1-13; example 49	1-12

☐ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

15 March 2002

Date of mailing of the international search report

28/03/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Stienon, P

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claims 1-2,5-6,9-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

-----

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/16502

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 0130331	A	03-05-2001	AU	1189201 A	08-05-2001
			WO	0130331 A2	03-05-2001
WO 0202094	A	10-01-2002	WO	0202094 A2	10-01-2002
WO 9517182	A	29-06-1995	US	5545636 A	13-08-1996
			AU	1339895 A	10-07-1995
			CA	2179650 A1	29-06-1995
			EP	0817627 A1	14-01-1998
			JP	9507066 T	15-07-1997
			WO	9517182 A1	29-06-1995
			US	5661173 A	26-08-1997
			US	5672618 A	30-09-1997
			US	5668152 A	16-09-1997
			ZA	9410139 A	20-06-1996

Form PCT/ISA/210 (patent family annex) (July 1992)